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DISCRIMINATION OF *LIRIOMYZA* SPECIES (DIPTERA: AGROMYZIDAE) USING ELECTROPHORESIS AND SCANNING ELECTRON MICROSCOPY

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Abstract.—Starch gel electrophoresis was used to distinguish three species of *Liriomyza* Mik, based on mobilities of four enzymes. Comparison of *L. brassicae* (Riley), *L. trifolii* (Burgess), and *L. sativae* Blanchard revealed different enzyme mobilities at three loci. *Liriomyza trifolii* and *L. sativae* were separated based on enzyme mobility differences at all four loci tested. Enzyme mobilities of conspecific strains of *L. trifolii* and *L. sativae* were not influenced by host feeding or geographic site of collection. Scanning electron micrographs revealed interspecific differences in length and spacing of mesonotal microsetae.

Leafmining flies in the genus *Liriomyza* Mik (Diptera: Agromyzidae) are serious pests of vegetable and ornamental crops in California. Specimens collected from celery in Ventura County, California, in 1980 were identified as a newly introduced species, *L. trifolii* (Burgess) (Trumble, 1981). This was the first record of an economically important infestation of *L. trifolii* on a vegetable crop in California. The introduction of this species has also affected the California ornamentals industry where *L. trifolii* has become the primary leafminer species damaging chrysanthemums (Parrella et al., 1981).

Prior to the introduction of *L. trifolii* to California, several leafminers had been documented as economic pests. *Liriomyza sativae* Blanchard was reported as a secondary pest of tomato capable of reaching injurious population levels when exposed to methomyl spray regimes (Oatman and Kennedy, 1976). *Liriomyza brassicae* (Riley) only sporadically causes serious damage to cruciferous crops and is considered of minor economic importance in California (Oatman and Platner, 1969). A fourth species, *L. huidobrensis* (Blanchard), is considered a pest of some ornamental plants and can attack legumes and other vegetable crops (Parrella, *in press*). Another economically important leafminer species, *L. trifoliearum* Spencer (as *L. pictella* (Thompson)), has been reported as a pest of alfalfa in California (Jensen and Koehler, 1970).

Fifteen *Liriomyza* species of economic importance in California have been reported, although only five species are currently recognized. The taxonomic confusion in the genus *Liriomyza* results from general morphological similarity and overlapping host ranges (Parrella, *in press*). Agromyzid nomenclature has recently been coordinated (Spencer, 1981) but misidentification may occur when morphological characters vary among individuals or when genitalia comparisons

are incorrectly interpreted. *Liriomyza trifolii, L. sativae,* and *L. brassicae* were included in this study because of morphological similarity, economic importance, and overlapping host ranges. *Liriomyza huidobrensis* and *L. trifoliearum* were not included because of gross morphological differences and restricted host range, respectively.

Within the last decade, gel electrophoresis of enzymes has become a suitable technique for discrimination of closely related taxa (Berlocher, 1979). Species in many insect genera have been identified using electrophoresis, including *Culex* Linnaeus (Mahon et al., 1976), *Speyeria* Scudder (Brittnacher et al., 1977), and *Drosophila* Fallén (Ayala and Powell, 1972). Berlocher (1980) developed a useful electrophoretic key to larvae, pupae, and adults of *Rhagoletis* Loew species.

Scanning electron microscopy has recently been used to delinate morphological structures for the description of new insect species (Boppré and Scherer, 1981; Bright, 1981). The purpose of our study was to determine whether gel electrophoresis or scanning electron microscopy could be utilized for separation of *Liriomyza* species.

Materials and Methods

Gel electrophoresis experiments.—*Liriomyza* spp. were obtained from the following sources:

Liriomyza trifolii: (1) A laboratory colony established on celery in December 1980 from an infestation occurring on celery in Ventura County, California. (2) Fresh market tomatoes in Ventura County, California. (3) Celery in South Bay, Florida.

Liriomyza sativae: (1) A laboratory colony established on squash in December 1980 from an infestation occurring on fresh market tomatoes in Irvine, California.

Liriomyza brassicae: (1) Broccoli in Irvine, California.

Liriomyza brassicae were difficult to collect and rear in the laboratory, and sample sizes were limited. All available *L. brassicae* were electrophoretically compared with *L. trifolii* and *L. sativae* on the same gels. Repeated comparisons of *L. trifolii* and *L. sativae* were performed since samples of these species were plentiful. Specimens were collected as larvae in infested plant material and held in an environmental chamber (27° ± 0.5°C, 65 ± 3% RH: LD = 14–10) for completion of development. After emergence, 48 hours were allowed for cuticular hardening and coloration before flies were transferred to glass vials and stored in liquid nitrogen. Numbers of individuals examined in each test have been reported in Tables 1 and 2 and Figs. 1–6.

Prior to homogenization for electrophoresis, individual adult flies were identified to species using mesonotal and vertical bristle characters described by Spencer (1965). Equal numbers of males and females were analyzed on each gel.

The starch gel electrophoresis technique described by Bush and Huettel (1972) was followed or slightly modified as described. Gels were formulated using 49.2 g Sigma hydrolyzed starch in 420 ml of gel buffer. Gel and electrode buffers were prepared as continuous tris citrate II (Selander et al., 1971), except pH was adjusted to 7.7 with additional citric acid. All four enzymes included in this study were separated on this buffer system. Gels were run at 4°C for five hours at 100 volts and ca. 70 milliamps. The enzyme systems examined were isocitrate dehydro-

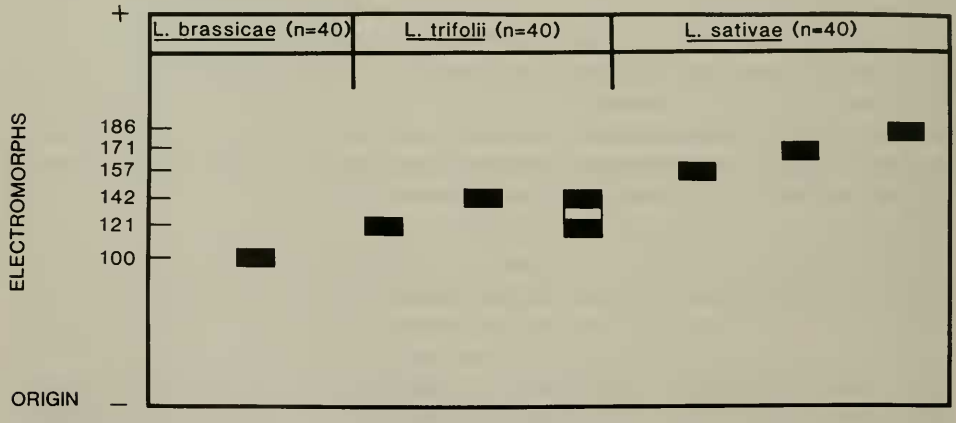
PHENOTYPE	100/100	133/133	120/120	133/133	120/133	160/160
FREQUENCY	.967	.033	.033	.933	.033	1.00
MIGRATION-DISTANCE(mm)	15	20	18	20	18–20	24

Fig. 1. Isocitrate dehydrogenase banding patterns in *Liriomyza brassicae, L. trifolii,* and *L. sativae.*

genase (IDH), aldehyde oxidase (AO), aconitase (ACON), and glucose-6-phosphate dehydrogenase (G-6-PDH). Stain and substrate buffer ingredients are as follows:

IDH–100 mg isocitric acid, 10 mg NADP, 100 mg $MgCl_2$, 15 mg MTT, and 5 mg PMS in 75 ml 0.1M tris HCl buffer (pH 7.5).

AO–10 mg NAD, 15 mg MTT, 5 mg PMS, and 2 ml benzaldehyde in 75 ml 0.1M tris HCl buffer (pH 8.4). Note: the glass stain dish must be tightly sealed or the benzaldehyde will diffuse and cause the AO reaction on nearby gels.



PHENOTYPE	100/100	121/121	142/142	121/142	157/157	171/171	186/186
FREQUENCY	1.00	.925	.025	.050	.100	.875	.025
MIGRATION-DISTANCE(mm)	7	8.5	10	8.5–10	11	12	13

Fig. 2. Aldehyde oxidase banding patterns in *Liriomyza brassicae, L. trifolii,* and *L. sativae.*

PHENOTYPE	142/142	152/152	100/100	106/106	100/106	116/116	123/123
FREQUENCY	.850	.150	.500	.400	.100	.850	.150
MIGRATION-DISTANCE(mm)	22	23.5	15.5	16.5	15.5–16.5	18	19

Fig. 3. Glucose-6-phosphate dehydrogenase banding patterns in *Liriomyza brassicae, L. trifolii,* and *L. sativae.*

ACON–100 mg cis-aconitic acid, 30 mg NADP, 100 mg $MgCl_2$, 15 mg MTT, 5 mg PMS, and 12 units isocitrate dehydrogenase in 75 ml 0.2M Na phosphate buffer (pH 8.0).

G-6-PDH–250 mg glucose-6-phosphate, 10 mg NADP, 100 mg $MgCl_2$, 20 mg MTT, and 5 mg PMS in 75 ml 0.1 M tris HCl buffer (pH 8.0).

Gel slices were incubated at 37°C until individual bands became distinct. One hour was sufficient for a complete stain reaction to occur for all enzymes. Band mobilities were measured from the sample origin. Electromorphs (terminology after King and Ohta, 1975) were assigned relative mobility values with 100 representing the most common electromorph. Electromorphs with faster or slower relative migration were assigned values greater or less than 100. In the interspecific comparisons with anodal migration (Figs. 1–5), 100 is assigned to the most common electromorph in the species with the slowest band migration. At the ACON locus (Fig. 6), the bands migrated cathodally and the 100 value is given to the most common electromorph in the fastest migrating species. Analysis of enzyme activity is presented as set forth by Berlocher (1980), where phenotypes are presented as combinations of electromorphs.

Scanning electron microscopy.—Specimens were sequentially immersed in increasing concentrations of alcohol up to 100%, then subjected to critical point drying, and examined with a JEOL model JSM-35C scanning electron microscope. The mesonotal regions of nine adult females of each species were photographed at 1200 magnification with a microscope voltage of 15 kV.

Results and Discussion

Intraspecific electrophoresis.—Differential host feeding in the larval stage did not prevent positive species identification since *Liriomyza trifolii* collected from different host plants produced similar electrophoretic banding patterns. When *L.*

Table 1. Biochemical similarity of *Liriomyza trifolii* from different hosts and geographic locations.

Locus	Migration[1] Distance (mm)	Phenotype Designation	Phenotype Frequency		
			Calif. Tomato	Calif. Celery	Florida Celery
Isocitrate dehydrogenase	18	95/95	.071	.045	.043
	19	100/100	.858	.910	.957
	18–19	95/100	.071	.045	—
(*N*)			(42)	(89)	(47)
Aldehyde oxidase	9	100/100	.750	.942	1.00
	11	122/122	.179	.029	—
	9–11	100/122	.071	.029	—
(*N*)			(28)	(70)	(44)
Aconitase[2]	−3	−100/−100	.952	.974	1.00
	−4	−133/−133	.024	.026	—
	−3–−4	−100/−133	.024	—	—
(*N*)			(42)	(77)	(35)
Glucose-6-phosphate dehydrogenase	15.5	94/94	.238	.250	.267
	16.5	100/100	.738	.694	.700
	15.5–16.5	94/100	.024	.056	.033
(*N*)			(42)	(72)	(30)

[1] Measured from sample origin to center of band.
[2] Migrates cathodally. A weak anodal band was not scored.

trifolii from the celery laboratory culture were compared with *L. trifolii* collected from fresh market tomatoes (Table 1), flies from both host plants were genetically similar and all but one phenotype were present in both strains at all loci. The only phenotype not shared by both the celery and tomato strains is the −100/−133 phenotype at the ACON locus found in one individual reared from tomato.

Similar results were found when *L. sativae*, reared for six months on squash in the laboratory, were compared with *L. sativae* collected from fresh market tomatoes (Table 2). At the IDH locus, all specimens were monomorphic and shared the 100/100 phenotype. The majority of individuals of both strains possessed the 100/100 phenotype at the other three loci. Rare phenotypes were also shared by both strains except for three individuals reared from tomato that demonstrated a 100/114 phenotype at the AO locus and three other individuals from tomato that exhibited a 79/129 phenotype at the same locus. Also, a −100/−250 phenotype was present in two individuals from tomato at the ACON locus that was not shared by those reared from squash.

Populations of *L. trifolii* collected from widely separated geographic locations demonstrated similar electrophoretic banding patterns (Table 1). Flies from celery in Florida were compared with a laboratory culture established from celery in Ventura County, California. Both strains shared the 100/100 phenotype in at least 91% of the specimens at the IDH, AO, and ACON loci. The Florida strain was less polymorphic than the California strain, producing fixed bands at the AO and ACON loci. Fewer individuals of both strains shared the 100/100 phenotype at the G-6-PDH locus when compared to other loci. This may have been caused by difficulty in scoring phenotypes due to lack of distinct borders for individual bands.

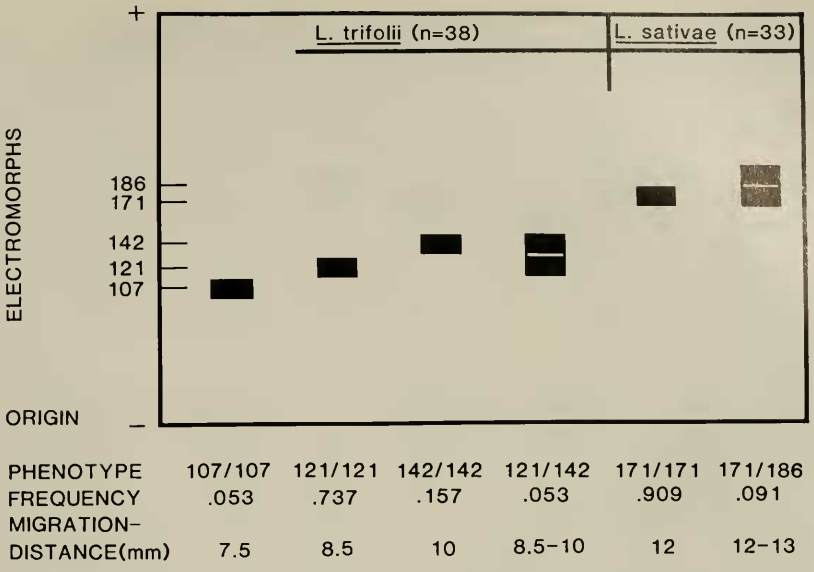


Fig. 4. Aldehyde oxidase banding patterns in *Liriomyza trifolii* and *L. sativae*.

However, relative mobility ranges of the Florida and California strains were the same (15.5–16.5 mm) at the G-6-PDH locus.

Interspecific electrophoresis.—*Liriomyza brassicae*, *L. trifolii*, and *L. sativae* demonstrated different enzyme mobilities at the IDH locus (Fig. 1), except for a

Table 2. Biochemical similarity of *Liriomyza sativae* reared from squash and tomato.

Locus	Migration[1] Distance (mm)	Phenotype Designation	Phenotype Frequency	
			Squash	Tomato
Isocitrate dehydrogenase	27	100/100	1.00	1.00
(*N*)			(45)	(45)
Aldehyde oxidase	14	100/100	.733	.533
	16	114/114	.267	.333
	14–16	100/114	—	.067
	11–18	79/129	—	.067
(*N*)			(45)	(45)
Aconitase[2]	−1	−100/−100	.822	.889
	−2.5	−250/−250	.178	.067
	−1–−2.5	−100/−250	—	.044
(*N*)			(45)	(45)
Glucose-6-phosphate dehydrogenase	21	95/95	.111	.267
	22	100/100	.800	.689
	23	105/105	.089	.044
(*N*)			(45)	(45)

[1] Measured from sample origin to center of band.
[2] Migrates cathodally. A weak anodal band was not scored.

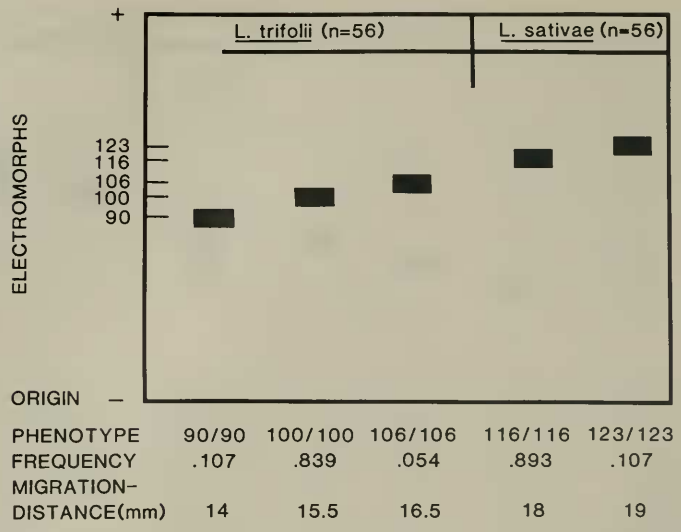


Fig. 5. Glucose-6-phosphate dehydrogenase banding patterns in *Liriomyza trifolii* and *L. sativae*.

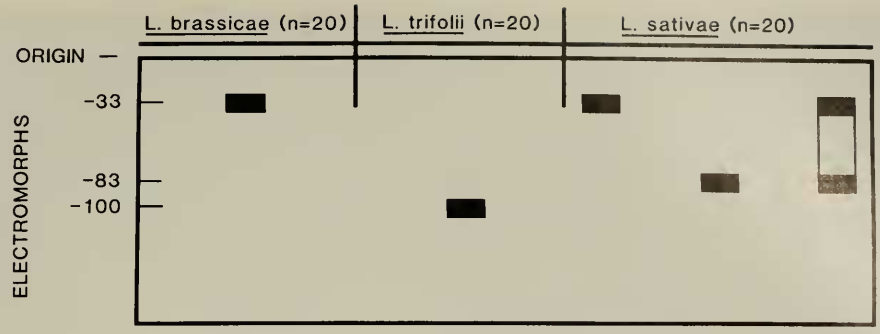
single *L. brassicae* which shared the most common 133/133 phenotype of *L. trifolii*. This individual may have been mislabeled or a flaw in the gel could have accounted for the faster band mobility. An additional 105 *L. trifolii* and 95 *L. sativae* demonstrated identical phenotypes to those shown for these species in Fig. 1. Except for the overlap resulting from the single specimen, the IDH locus proved suitable for separation of the three species.

Enzyme mobility differences between the three species were also demonstrated at the AO and G-6-PDH loci (Figs. 2, 3). The relative mobilities were the same for AO and IDH, where *L. brassicae, L. trifolii,* and *L. sativae* had increasing mobility rates, respectively. Banding patterns for enzymes at the G-6-PDH locus were recognizably different: *L. brassicae* migrated fastest and *L. trifolii* slowest.

An additional 38 *L. trifolii* were compared to 33 *L. sativae* at the AO locus (Fig. 4). Three *L. sativae* demonstrated a new 171/186 phenotype not present in Fig. 2. Five of 40 *L. sativae* in Fig. 2 possessed 157/157 or 186/186 phenotypes not seen in the 33 *L. sativae* in Fig. 4. Two of 38 *L. trifolii* in Fig. 4 possessed a rare 107/107 phenotype not present in the 40 *L. trifolii* in Fig. 2.

Fig. 5 represents the G-6-PDH locus for an additional 56 individuals each of *L. trifolii* and *L. sativae*. A new 90/90 phenotype not seen in Fig. 3 is present in 6 *L. trifolii* in Fig. 5. The 100/106 phenotype seen in 2 *L. trifolii* in Fig. 3 is not present in any of the 56 *L. trifolii* in Fig. 5. The 56 *L. sativae* in Fig. 5 and the 20 *L. sativae* in Fig. 3 had identical phenotypes.

It is possible that unique individuals do not represent rare phenotypes but that they were scored incorrectly or their bands altered by gel defects. Since one or only a few specimens often yield adequate data for the description of an entire species (Avise, 1975), these rare phenotypes can be ignored. In any case, rare phenotypes accounted for no band overlap between species and thus did not prevent accurate discrimination.



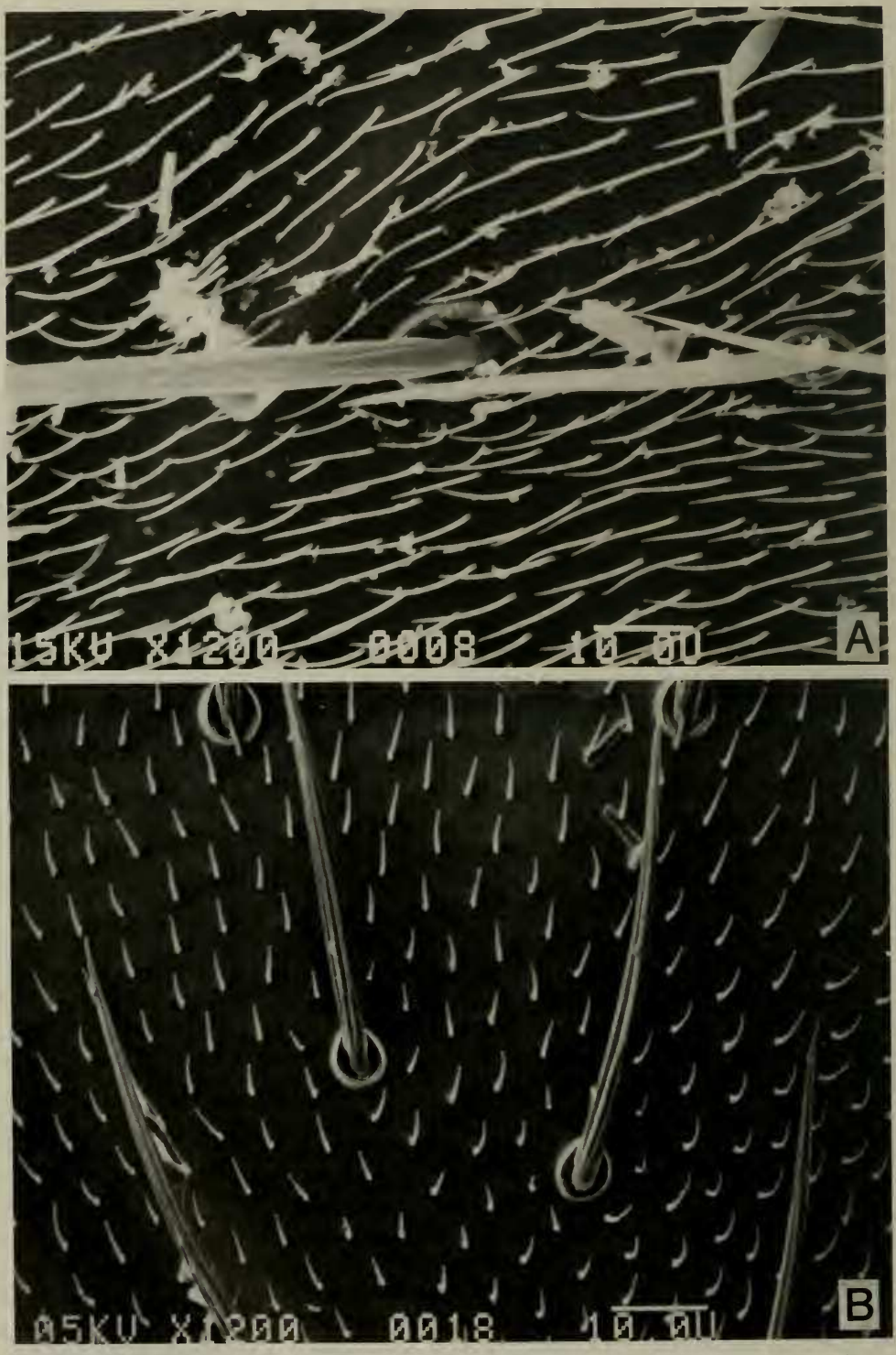
PHENOTYPE	-33/-33	-100/-100	-33/-33	-83/-83	-33/-83
FREQUENCY	1.00	1.00	.750	.100	.150
MIGRATION-DISTANCE(mm)	-1	-3	-1	-2.5	-1-2.5

Fig. 6. Aconitase banding patterns in *Liriomyza brassicae, L. trifolii,* and *L. sativae.*

ACON was not diagnostic for all three species since *L. brassicae* and *L. sativae* shared a common −33/−33 phenotype (Fig. 6). This locus was suitable for discrimination of *L. brassicae* and *L. sativae* from *L. trifolii.* An additional 53 *L. trifolii* and 48 *L. sativae* had identical phenotypes to those presented for these species in Fig. 6.

At most loci, the frequency of heterozygotes was lower than predicted by Hardy-Weinberg equilibrium. For example, *L. sativae* reared from squash possessed homozygous phenotypes at the AO locus with frequencies of 0.73 and 0.27, with no heterozygotes (Table 2). The presence of homozygotes with absence of heterozygotes can be explained by the Wahlund effect (Ferguson, 1980). This occurs when a sample is actually a mixture of two fully or partially isolated populations possessing different allelic frequencies. The frequencies of 0.73 and 0.27 suggest that the *L. sativae* culture consists of two such populations present in a 3:1 ratio. Sex-linkage of the enzyme systems, or active selection in a population, may also result in a deficiency of heterozygotes (Ferguson, 1980). Nonetheless, electrophoretic discrimination of the three species of *Liriomyza* is valid since the range of enzyme mobility for each species is separable at several loci.

Scanning electron microscopy.—A morphological character used in separation of *L. sativae* and *L. trifolii* is the shiny black vs. mat-grey mesonotum (Spencer, 1965). Scanning electron micrographs of the mesonota of *L. brassicae, L. sativae,* and *L. trifolii* were taken to determine the ultrastructure responsible for these differences. A dense covering of microsetae in *L. trifolii* (Fig. 7A) is responsible for the mat-grey appearance of its mesonotum. Large areas of exposed cuticle give *L. sativae* (Fig. 7B) and *L. brassicae* (Fig. 7C) the shiny black appearance noted by Spencer. *Liriomyza brassicae* appears to have microsetae that are curved more than those of *L. sativae.* This curvature may be due to slightly different orientation of the flies to the electron beam, since curved microsetae were not evident on all *L. brassicae* photographed. Although *L. trifolii* can be separated



Figs. 7A, 7B. Scanning electron micrographs of mesonotum. A, *Liriomyza trifolii.* B, *L. sativae.*

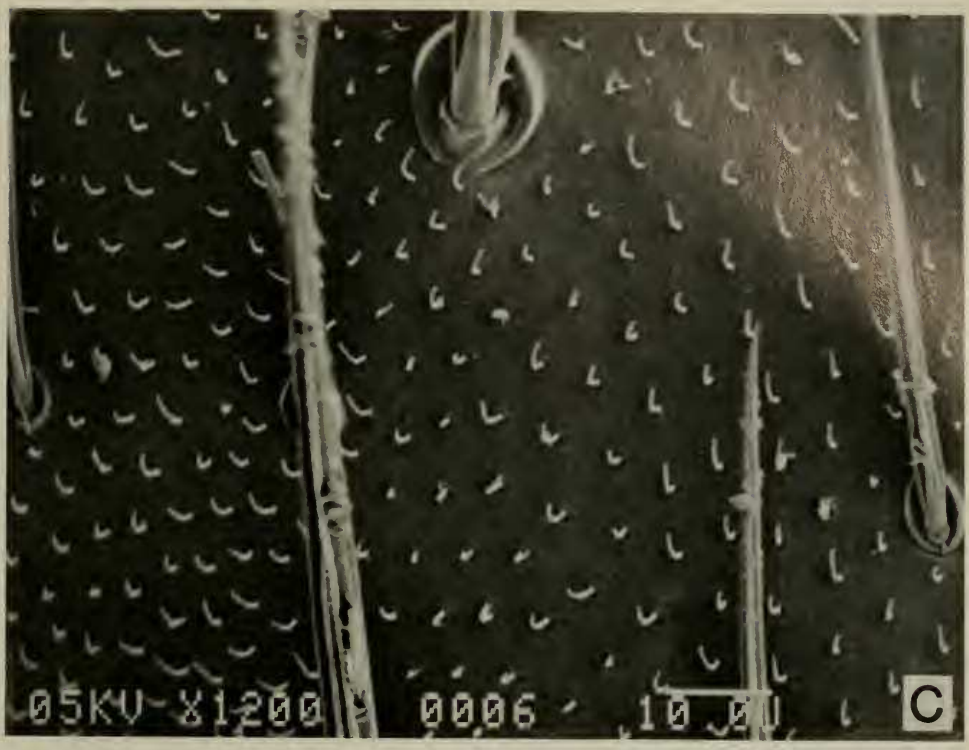


Fig. 7C. Scanning electron micrographs of mesonotum, *Liriomyza brassicae.*

from *L. sativae* and *L. brassicae* by electron microscopy, this technique is not suitable for discrimination between *L. sativae* and *L. brassicae.*

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